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FMUP FACULDADE DE MEDICINA UNIVERSIDADE DO PORTO

MESTRADO INTEGRADO EM MEDICINA

2017/2018

Ema Catarina Monteiro dos Santos

Fluorescence-guided resection with 5-aminolevulinic acid: implications, complications and learning after 100 cases

março, 2018

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Ressecção guiada por fluorescência com ácido 5-aminolevulinico: implicações, complicações e aprendizagem após 100 casos

Mestrado Integrado em Medicina

Área: Neurocirurgia

Tipologia: Dissertação

Trabalho efetuado sob a Orientação de:

Paulo José Campos Linhares Vieira

Trabalho organizado de acordo com as normas da revista:

Acta Neurochirurgica

março, 2018

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Projecto de Opção do 6º ano – DECLARAÇÃO DE REPRODUÇÃO

NOME

Ema Catarina Monteiro dos Santos

NÚMERO DE ESTUDANTE | E-MAIL

201202274 | emamsantos@gmail.com

DESIGNAÇÃO DA ÁREA DO PROJECTO

Neurocirurgia

TÍTULO DISSERTAÇÃO

Fluorescence-guided resection with 5-aminolevulinic acid: implications, complications, and learning after 100 cases

ORIENTADOR

Paulo José Campos Linhares Vieira

COORIENTADOR (se aplicável)

-

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Faculdade de Medicina da Universidade do Porto, 19/03/2018

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Fluorescence-guided resection with 5-aminolevulinic acid: implications, complications and learning after 100 cases

Ema Santos[1], Bruno Carvalho[1,2], Rui Vaz,[1,2,3] Paulo Linhares[1,2,3]

[1] Faculty of Medicine, Oporto University, Oporto, Portugal

[2] Department of Neurosurgery, Hospital São João, São João Hospital Centre, Oporto, Portugal

[3] Neurosciences Center, Hospital CUF Porto, Oporto, Portugal

Corresponding author:

Ema Catarina Monteiro dos Santos

Faculty of Medicine, Oporto University, Oporto, Portugal

Alameda Prof. Hernâni Monteiro, 4200-319 Porto

E-mail: emamsantos@gmail.com

Abstract

Background: 5-aminolevulinic acid (5-ALA) has demonstrated to be a safe and useful tool for real-time intraoperative identification of tumor tissue, leading to extensive resections which are associated with increased survival for patients with high-grade gliomas. Although tumors in eloquent areas can constrain resection due to higher risk of neurological deficits, this should not contra-indicate 5-ALA judicious use.

Objective: To evaluate the impact of resection in progression-free survival (PFS) and overall survival (OS), the postoperative complications and learning after the first 100 cases of 5-ALA fluorescence-guided resection in a tertiary referral hospital.

Methods: A retrospective study of the first 100 cases of newly diagnosed brain lesions submitted to fluorescence-guided surgery with 5-ALA, from 2012 to 2016. Only glioblastoma patients were considered for the statistical analysis. We analyzed the influence of 5-ALA in the extent of resection considering tumor in eloquent and non-eloquent location, post-operative neurological deficits, PFS and OS.

Results: Gross total resection (GTR) was achieved in 73% of patients with tumors in non-eloquent areas and in 43,4% in eloquent areas. Partial resection was superior in tumors located in eloquent areas (34,0%vs13,5%). There were no significant differences in postoperative neurological deficits amongst distinct extents of resection (p=0,228) or tumor location (p=0,670). Mean PFS for all group was 9,73±1,19 months and mean OS for all group was 16,27±1,39 months. GTR>90% and the non-eloquent area of the tumor were correlated with a significant increase in PFS but the extent of resection was the only independent factor for PFS in multivariate analysis. Regarding OS, no independent prognosis factor was established in the multivariate analysis.

Conclusion: 5-ALA is an important tool in glioblastoma surgery, contributing for GTR and, consequently, to increase PFS. Although its use has not raised neurological deficits, it must be carefully used in eloquent located tumors.

Key-words: 5-aminolevulinic acid; fluorescence-guided resection; glioblastoma; extent of resection;

Introduction

The extent of resection has been recognized as an established prognostic factor in the treatment of glioblastoma (GBM) [13,1], being the only one potentially modifiable. Complete resection of enhancing tumor is linked to an improvement in survival [2,17,16,13,21], acting synergistically with optimized medical treatment [18].

Multiple techniques were developed in order to achieve a better identification of the tumor from adjacent normal brain, being fluorescence-guided resection with 5-aminolevulinic acid (5-ALA) one of the most innovative.

5-ALA is a metabolic precursor in the heme pathway and can be used as an oral prodrug that leads to the synthesis and accumulation of fluorescent porphyrins, mainly protoporphyrin IX, in high-grade glioma cells [20,19,15].

Several studies have showed that 5-ALA intraoperative fluorescence has a very high sensitivity, specificity and positive predictive value (PPV) for malignant tumor tissue [22,15,7,26,23,11,8,10]. Additionally, there are two patterns of fluorescence: “strong”, associated with solid and proliferating tumor and “weak” corresponding to infiltrating tumor with both tumor and normal cells. These patterns correlate with sensitivity, specificity and PPV, and has been found that these values can reach 100% in areas with “strong” fluorescence [23,7,11,12,10], confirming the remarkable diagnostic precision of 5-ALA.

Introduced by Stummer et al.[22], it has since demonstrated to be a secure and accurate tool for real-time intraoperative identification of tumor cells, leading to extensive tumor removal and an increase in survival for patients with high-grade gliomas[20,19,5]. It remains inconsistent in the literature if the increased survival expresses progression-free survival or overall survival.

However, it cannot be ignored that more radical resections carry more risks of neurological damages [24], particularly in tumors near eloquent areas. The aim of using 5-ALA in eloquent areas is not to achieve gross total resection (GTR) but increase the accuracy for tumor cells. While some authors only use immunofluorescence in cases of expected GTR, our understanding is that 5-ALA also helps in defining tumor cells even in cases only suitable for partial resections (PR).

The goals of the present study are to evaluate the impact of the extent of tumor resection in progression-free survival and overall survival, the possible

post-surgical complications and the lessons learned after the first 100 cases using fluorescence-guided resection with 5-ALA.

Materials and Methods

Patient population

The data used in this study was obtained from clinical records of the first 100 cases submitted to fluorescence-guided surgery with 5-ALA, from May 2012 until February 2016 in a European Union University Hospital. All medical record reviews were conducted with approval from the Local Ethical Committee. For this type of study formal consent is not required.

The criteria for the use of 5-ALA were newly diagnosed brain lesions with MRI suggestive of high grade malignant glioma in patients eligible for open surgery, according to their clinical status.

Tumors were categorized as located in eloquent and non-eloquent areas. The primary motor cortex (precentral gyrus), primary somatosensory cortex (postcentral gyrus), occipital visual cortex, insula, corpus callosum and the operculum area in the frontal, parietal and temporal left lobes were considered eloquent areas.

From a total of 100 patients submitted to surgery with 5-ALA, 90 were diagnosed with GBM after histopathological confirmation. In the remaining 10 patients, 3 were diagnosed with anaplastic astrocytoma and 7 with different histological types. Due to the diversity of diagnoses, patients who were not diagnosed with GBM were excluded in the further analysis. **Table 1** shows the patient and tumor baseline characteristics.

Technical principals

5-ALA (Gliolan, Medac, Wedel, Germany) oral solution was administered to patients at 20mg/kg bodyweight, 2-4h before general anesthesia. Dexamethasone was also provided to all patients at least two days before surgery up to the postoperative MRI, less than 72h after surgery. It has been reported that dexamethasone use improves fluorescence.

The tumor was resected using a surgical microscope (*Zeiss OPMI Pentero 900*® or Leica OHS®) that is able to switch from white conventional light (no fluorescence visible) to violet-blue light (BL400) whenever desired by the

neurosurgeon, allowing fluorescent tumor tissue identification. The goal of the surgery was to remove safely all the fluorescent tissue visible, considering possible neurological deterioration when near to eloquent areas. Other intraoperative diagnostic tools, such as neuronavigation and awake monitoring were used.

The extent of resection was assessed by comparison of preoperative MRI with postoperative MRI less than 72h after surgery and then classified as GTR, subtotal resection (STR) or PR. GTR was defined as absence of residual contrast in post-op MRI; the resection was considered subtotal when was superior to 90% of the volume but not complete; PR included less than 90% of the tumor volume removed.

Treatment and follow up

After diagnostic confirmation of GBM, patients were referred to a neuro-oncology multidisciplinary team (MDT) meeting to evaluate their clinical conditions to pursue further treatment.

From the patients diagnosed with GBM, 76 started the Stupp protocol, radiotherapy with concomitant and adjuvant temozolomide[25], one patient with recurrent brain lesion started second-line chemotherapy after surgery and six patients were referred to palliative care. Seven patients were not submitted to any treatment after surgery due to their condition (n=6) or treatment refusal (n=1) and were submitted to best supportive care.

Progression was defined as an increase in residual tumor volume, the occurrence of a new tumor lesion or deterioration in the clinical status, according to RANO criteria. After progression, the neuro-oncology MDT would decide whether to start second-line chemotherapy, re-operate or refer to palliative chemotherapy according to the clinical and functional condition of each patient.

Statistical analysis

The statistical analysis was performed using IBM SPSS® (Statistical Package for Social Sciences) version 24. Statistical significance was set at $p<0,05$. It was conducted a descriptive analysis of all variables.

To test the possible effect of multiple factors like age, sex, preoperative KPS and area of the tumor (eloquent/non-eloquent) in the extent of resection, separately Chi-square tests were performed. It was also accessed the influence of the resection on post-operative neurological deficits with a Chi-square test.

Progression-free survival and overall survival were defined as the time between surgery and progression or time of death, respectively. To identify factors influencing survival, Kaplan-Meier survival curves were constructed individually for sex, age, preoperative KPS, tumor eloquence and extent of resection. For survival analysis, the extent of resection was grouped in gross total/subtotal (tumor removal superior to 90%) and partial resection due to the quantitative difference of tumor not removed.

It was also analyzed if the extent of resection suffered a variation through with time, comparing the extent of resection of the first 45 patients with GBM submitted to 5-ALA guided-resection with the last 45 patients.

Results

Resection

Patients were divided in two groups: tumors in non-eloquent areas (n=37) and tumors in eloquent areas (n=53). It was observed a significant difference in the extent of resection between the groups (p=0,019) (FIG1). GTR was accomplished in 73% of patients with non-eloquent tumors, 13,5% had STR and 13,5% PR, which means that it was obtained a resection >90% in 86,5% of patients. Concerning tumors in eloquent areas, resections >90% were achieved in 66% being GTR 43,4% of the patients, 22,6% had STR of the tumor and 34,0% had PR. Even though in both groups GTR was achieved more often than subtotal or partial, these results demonstrate a bigger proportion of gross total removal in tumors in non-eloquent areas and a higher percentage of PR in eloquent areas, what was expected since the aim of resection in eloquent areas were the maximal safe resection and not GTR.

Factors which could have influenced the extent of resection such as age, sex, preoperative KPS and time within series (Table 2) were also considered. The analysis demonstrated that sex, age and preoperative KPS could not prove to be significant prognosis factors even though it appears that a GTR is accomplished more frequently in younger patients or with KPS score superior to

70. The extent of resection did not suffer a variation when comparing the first 45 patients submitted to 5-ALA guided resection with the latter 45 patients.

Safety

Post-operative complications were divided in de novo epilepsy (n=2), hemorrhagic/ ischemic events (n=3), infection (n=2) and new/aggravation of focal deficit (n=20). The majority of patients (70%) have not developed any complication. KPS score 1 month after surgery remained identical to pre-op KPS score (median=90).

From all the post-operative complications detected, it was considered that only new/aggravation of focal deficit could result from the use of 5-ALA since its main goal is to improve the extent of resection, which can ultimately lead to neurological deterioration. It was also considered if focal deficits could vary with the area of the tumor (eloquent/non-eloquent) as eloquent areas would be more prone to subsequent deficits.

After analysis, no difference in post-op focal deficit was found amongst tumors in eloquent areas comparing with non-eloquent areas (p=0,670). Moreover, the neurological deficits have not diverged when compared between gross total, subtotal or partial resection (p=0,228).

Survival

Mean overall survival for all group was 16,27 $\pm$ 1,39 months and mean progression-free survival for all group was 9,73 $\pm$ 1,19 months.

For the analysis of survival, potential prognosis factors were evaluated such as sex, patient age, preoperative KPS score, localization of tumor and the extent of resection.

Patient age inferior to 65 years old (<65 = 18,01$\pm$1,57 months vs $\geq$65 =13,22$\pm$2,31 months; p=0,043) and a preoperative KPS score superior to 70 (KPS>70=17,36$\pm$1,54 months vs KPS$\leq$70=10,13$\pm$2,00 months; p=0,028) were significantly associated with longer overall survival. Extent of resection (GTR>90%=17,52$\pm$1,68 months vs PR=13,130$\pm$2,62 months; p=0,107) and tumor area (eloquent=13,71$\pm$1,32 months vs non-eloquent=19,32$\pm$2,41 months; p=0,077) failed to demonstrate a significant association, even though it appears to exist a tendency for improvement of overall survival when the resection is

>90% or the tumor is located in a non-eloquent area **(FIG. 2)**. No independent prognosis factor for overall survival was established in the multivariate analysis.

Furthermore, a complete resection (GTR>90%=11,33±1,54 months vs PR=5,30±0,93 months; p=0,001), the non-eloquent area of the tumor (eloquent=7,15±0,81 months vs non-eloquent=13,58±2,59 months; p=0,007) and once again, the preoperative KPS score superior to 70 (KPS>70=10,48±1,37 months vs KPS≤70=5,49±1,10 months; p=0,028) showed a significant increase in progression-free survival. The difference in patient age was not statistically significant in progression-free survival (<65=9,78±1,17 months vs ≥65=9,22±2,25 months; p=0,308) **(FIG 3).** Multivariate analysis was performed, showing that resection superior to 90% is the only independent factor for progression-free survival (p=0,030)

There were no differences in overall survival (male=17,07±2,03 months vs female=14,83±1,57months; p=0,590) or progression-free survival (male=9,66±1,50 months vs female=9,19±1,51months; p=0,820) related with gender.

Discussion

Glioblastoma is the most common malignant primary brain tumor and it is associated with a very poor prognosis. Tumor removal has a major role in treatment, being proven that the extent of resection is an important prognosis factor for patient survival [21,17,16,13,2,1]. This led to the development of 5-ALA with the goal of achieving more radical resections thus improving survival. [22,21,20]

Multiple studies revealed GTR rates superior to 60% using 5-ALA, representing a major improvement from “white-light” surgery [20,19,8,7,3]. Although 5-ALA is used mostly in patients were the goal is GTR, we used it in all patients with glioblastoma regardless of the expected extent of resection. Because of this, we divided our results in two different groups, the one that is expected a GTR and the group which STR was the aim because of an eloquent area is present. Tumors in eloquent areas have a particular importance due to increased risk of neurological damage and can substantially limit the resection. One must not forget the importance of the quality of life in patients with such a short life expectancy and improving resection does not fulfill its goal if

accompanied with new or aggravation of neurological deficits and can even decrease overall survival.[14] These leads to differences in the extent of resection when comparing tumors in eloquent and non-eloquent areas.

In the present study, resection >90% of tumor volume was observed in 86,5% and GTR in 73% of patients with tumors located in non-eloquent areas, in contrast with only 66% with resection >90% and 43,4% with complete absence of contrast of tumors located near an eloquent area. On the other hand, the proportion of PR and STR was superior in tumors adjacent to eloquent areas. These results reflect a careful approach to the use of 5-ALA specially in eloquent areas, not removing all the fluorescent tissue that is observed intraoperatively to prevent further neurological damages. Other intraoperative diagnostic tools were used, but in order to achieve better results in resection it has been suggested the use of intraoperative monitoring in combination with 5-ALA in all tumors located in eloquent areas. [9,6]

It was detected that the extent of resection did not suffer a modification when comparing the first 45 patients submitted to 5-ALA guided resection with the latter 45 patients. This lack of variation through time can be rationalized by the fact that few surgeons started performing this type of surgery progressively and their diverse learning curves.

Regarding safety, some authors described a temporarily neurological impairment in patients with more radical resections[24]. We have not experienced differences in postoperative focal deficit amongst tumors in eloquent areas comparing with non-eloquent areas or when compared between GTR, STR or PR, although this can be consequence of the careful approach and higher proportion of PR and STR in eloquent tumors.

Our mean overall survival for all group was 16,27 $\pm$ 1,39 months and mean progression-free survival for all group was 9,73 $\pm$ 1,19 months which is concordant with the results in the literature [11] even with lower resection rates.

Exploring overall survival, we observed that younger ages and a good preoperative status were significantly associated with longer overall survival in univariate analysis but not in multivariate analysis. Additionally, it appeared to exist a non-significant tendency for improvement of overall survival when the tumor is located in a non-eloquent area or the resection is >90%, what would be consistent with previous results referring a survival benefit in resections superior

to 70% [17,2]. In spite of that, OS could be a controversial end-point owing to the difficulty to control treatments and interventions after progression. In fact, Bloch et al.[4] stated that the extent of resection at recurrence can be an essential predictor of overall survival. The differences in post-recurrence treatments or re-operations were outside the sphere of this work but could explain the lack of statistical significance in respect of resection and OS.

A significant improvement in progression-free survival was noted with GTR>90%, the non-eloquent area of the tumor and a favorable preoperative status. In multivariate analysis, resection superior to 90% was the only independent factor. PFS was also the clinical benefit described by Stummer et al.[20]

Concerning limitations, the retrospective design of the study and the small sample size are important factors and can explain the lack of statistical significance in some variables. Further prospective studies with larger samples are needed to help recognize statistical significance in all the analysis a tendency was acknowledged and to improve the evidence level for the use of 5-ALA guided resection.

Conclusions:

5-ALA is an important tool in glioblastoma surgery, contributing for GTR and, consequently, to duplicate PFS from 5,30 to 11,33 months. Although its use has not raised neurological deficits, it must be carefully used in eloquent tumors. Our data were according to the literature, showing better results when 5-ALA were used in tumors in non-eloquent areas where the aim is to achieve gross total resection.

Conflict of Interest:

The authors declare that they have no conflict of interest.

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Table 1. Patient and tumor characteristics		n=100
Gender	Male	52
	Female	48
Age	Range	26-80
	Mean	58,73 ±11,91
	Median	61
Pre-operative KPS	Range	20-100
	Median	90
	>70	84
	≤70	16
Tumor location	Eloquent	58
	Non-eloquent	42
Histology	GBM	90
	Anaplastic astrocytoma	3
	Anaplastic oligodendroglioma	1
	Anaplastic ependymoma	1
	Grade II astrocytoma	1
	Grade II oligodendroglioma	1
	Grade III meningioma	1
	Necrosis	1
	Vasculitis	1
Treatment after surgery: GBM	Stupp protocol	76
	Bevacizumab+irinotecan	1
	Palliative care	6
	Best supportive care	7

Table 2. Cross tabulation

		Resection				
		Partial (n=23)	Subtotal (n=17)	Gross total (n=50)	Total (n=90)	**p**
Sex	Male	13	5	29	47	0,112
	Female	10	12	21	43	
Age	<65	11	10	35	56	0,183
	≥65	12	7	15	34	
KPS pre-op	≤70	7	3	5	15	0,093
	>70	16	14	45	75	
Patients	1-45	13	9	23	45	0,680
	45-90	10	8	26	45	

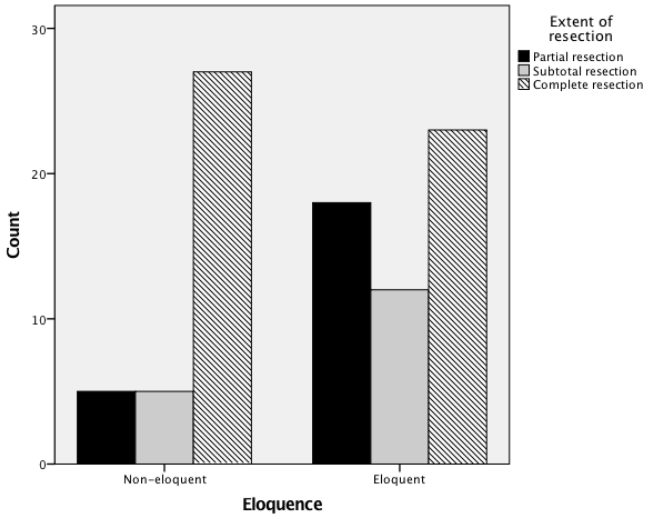


FIG 1. Different extents of resection stratified by eloquent/non-eloquent area of tumor (p=0,019)

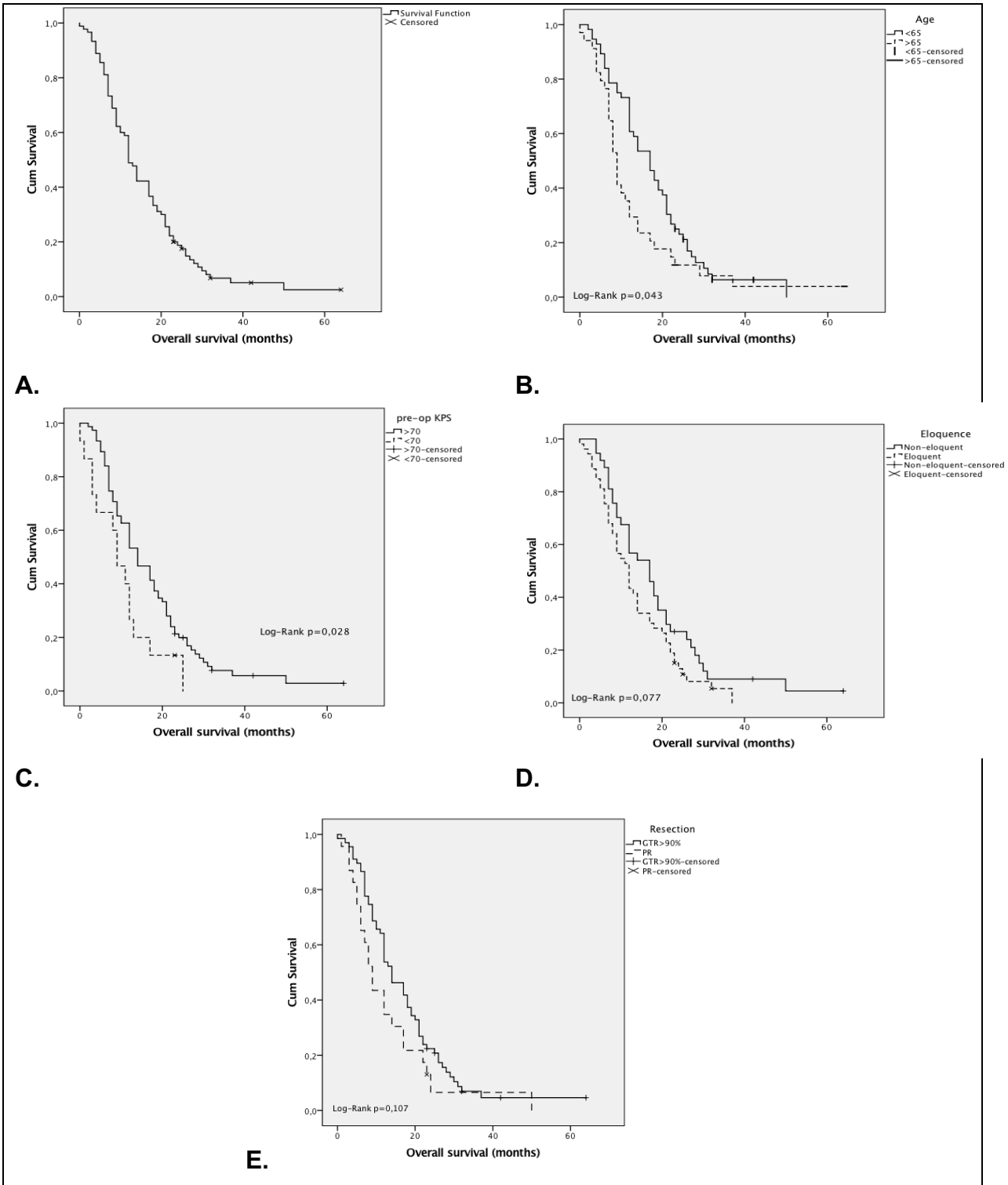


FIG. 2. Graphs showing Kaplan-Meier overall survival curves. A. Overall survival – 16,27 ± 1,39 months **B. Survival stratified by age** – <65 = 18,01±1,57 months; ≥65 =13,22±2,31 months. **C. Survival stratified by preoperative KPS score** - KPS>70=17,36±1,54 months; KPS≤70=10,13±2,00 months. **D. Survival stratified by tumor area (eloquent/non-eloquent)** – eloquent =13,71±1,32 months (median=12,00±1,55); non-eloquent =19,32±2,41 months. **E. Survival stratified by extent of resection** – GTR>90%=17,52±1,68 months; PR =13,130±2,62 months.

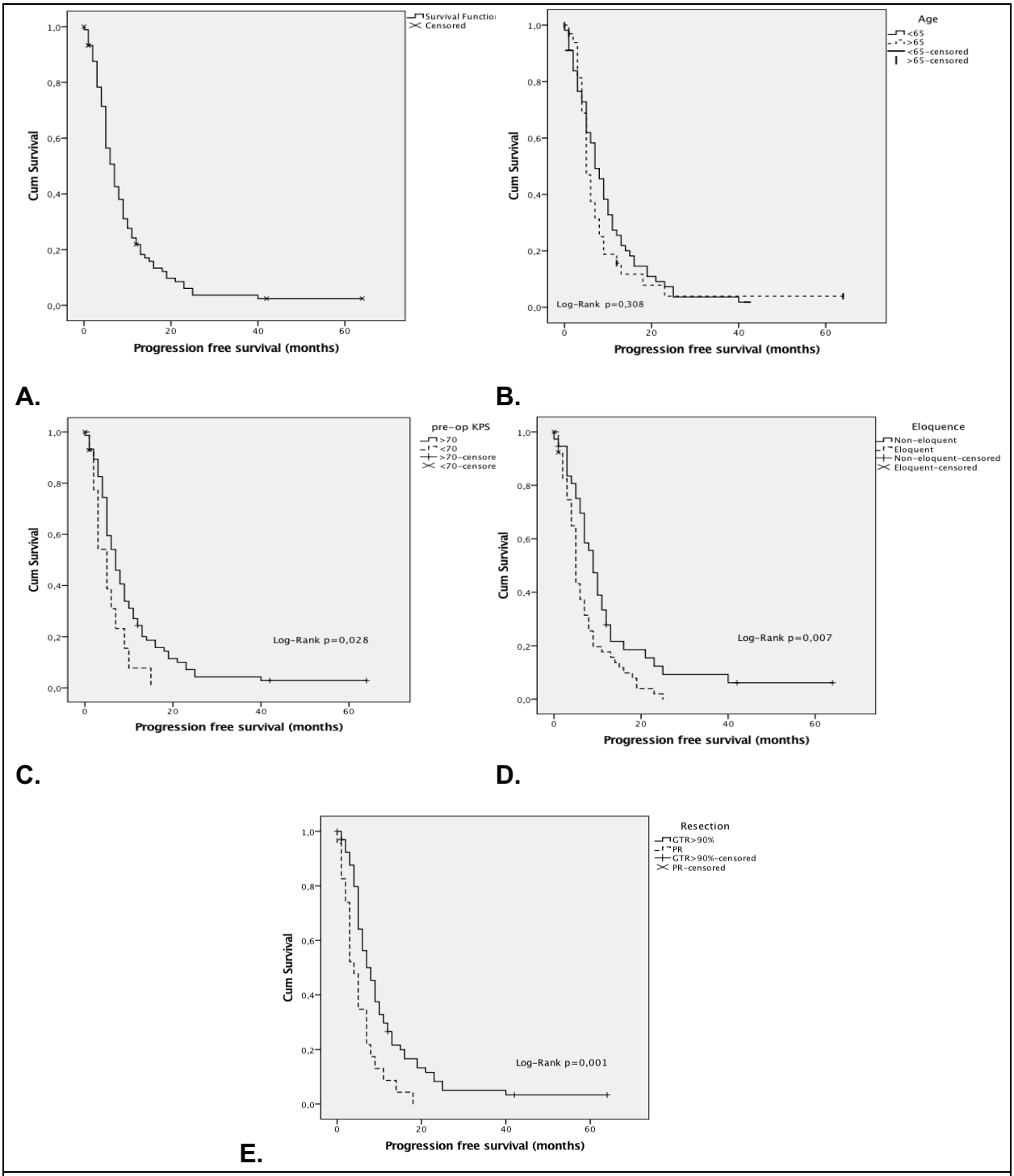
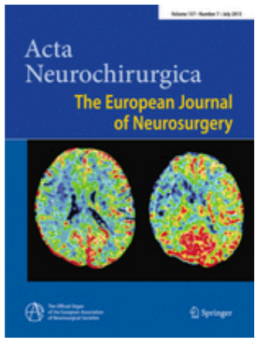


FIG. 3. Graphs showing Kaplan-Meier progression-free survival curves. A. Progression-free survival – 9,73 ± 1,19 months. **B. Survival stratified by age** – <65=9,78±1,17 months; ≥65=9,22±2,25 months. **C. Survival stratified by preoperative KPS score** - KPS>70=10,48±1,37 months; KPS≤70=5,49±1,10 months. **D. Survival stratified by tumor area (eloquent/non-eloquent)** – eloquent=7,15±0,81 months; non-eloquent=13,58±2,59 months. **E. Survival stratified by extent of resection** – GTR>90%=11,33±1,54 months; PR=5,30±0,93 months.



Acta Neurochirurgica

The European Journal of Neurosurgery

Editor-in-Chief: Tiit **Mathiesen**

ISSN: 0001-6268 (print version)
ISSN: 0942-0940 (electronic version)

Journal no. 701

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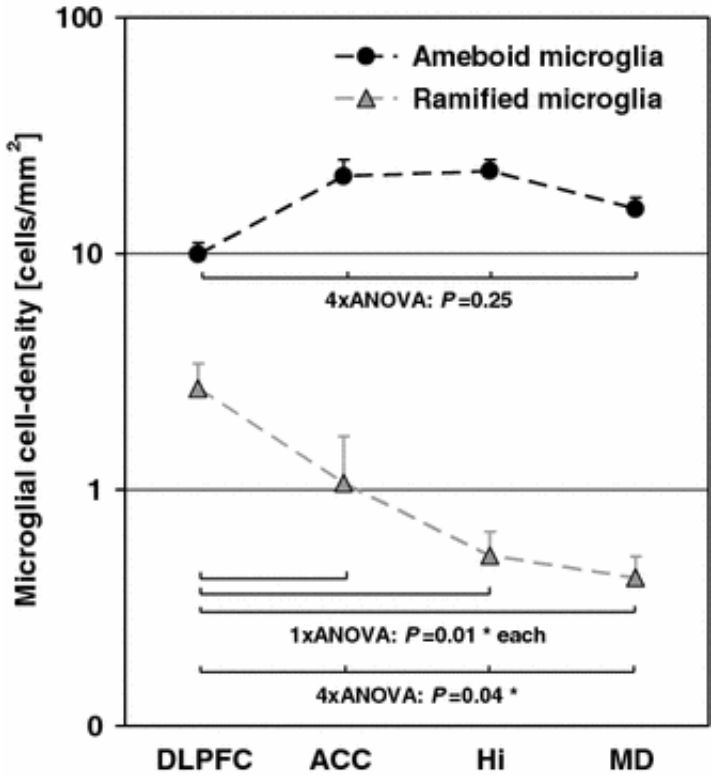

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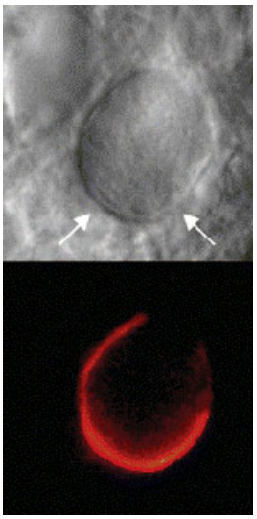

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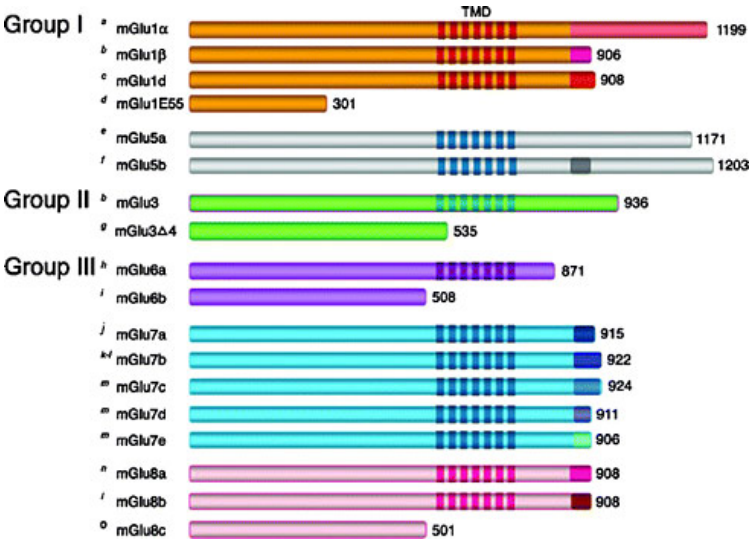

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